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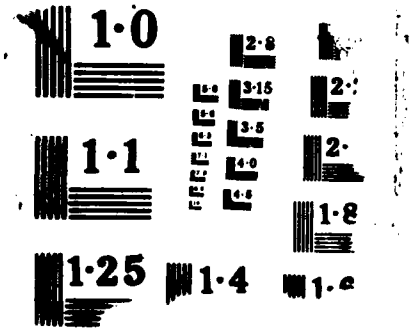
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MIDWINTER CONFERENCE OF IMMUNOLOGISTS

P R O C E E D I N G S

THE TWENTY-SEVENTH MIDWINTER CONFERENCE OF IMMUNOLOGISTS
January 23-26, 1988
Asilomar Conference Center
800 Asilomar Avenue, Pacific Grove, California

TITLE: ANTIGEN PROCESSING AND T CELL ACTIVATION

CHAIRPERSONS:
Dr. Carol Cowing
Medical Biology Institute, La Jolla, CA
Dr. Howard Grey
National Jewish Center for Immunology and Respiratory Medicine, Denver, CO
Dr. Arthur Weiss
University of California, San Francisco, CA

SESSION I: INTRACELLULAR COMPARTMENTS AND PROTEIN TRANSPORT
Chairperson: Dr. Peter Cresswell
Duke University Medical Center, Durham, NC

Speakers:

DR. FRANCES M. BRODSKY
University of California, San Francisco, CA
"The mechanics of intracellular traffic."

DR. MARTIN D. SNIDER
Case Western Reserve School of Medicine, Cleveland, OH
"Pathways followed by cell surface glycoproteins after endocytosis."

DR. PHILLIP D. STAHL
Washington University School of Medicine, St. Louis, MO
"Uptake and processing of proteins in macrophage endosomes."

DR. PETER CRESSWELL
Duke University Medical Center, Durham, NC
"Processing and transport of MHC molecules."

SESSION II ANTIGEN PROCESSING AND ANTIGEN-MHC ASSOCIATIONS
Chairperson: Howard M. Grey

Speakers:

HOWARD M. GREY
National Jewish Center for Immunology and Respiratory Medicine, Denver, CO
"Characteristics of peptides that are required for interaction with Ia and recognition by T cells"

MALCOLM L. GEFTER
Massachusetts Institute of Technology, Cambridge, MA

"Synthetic peptides as tools for analysis of class II restricted immune responses"

HARDEN M. McCONNELL
Stanford University, Stanford, CA
"Kinetic control of antigen presentation"

THOMAS J. BRACIALE
Washington University, St. Louis, MO
"Antigen processing and presentation required for class I MHC restricted T cell recognition"

SESSION III ANTIGEN-PRESENTING CELLS IN IMMUNITY AND TOLERANCE
Chairperson: Carol Cowing

Speakers:

CAROL COWING
Medical Biology Institute, La Jolla, CA
"Antigen-presenting cells in MHC class II transgenic mice"

JONATHAN SPRENT
Scripps Clinic and Research Foundation, La Jolla, CA
"The thymus and T cell tolerance induction"

JOHN G. TEW
Medical College of Virginia, Richmond, VA
"The role of follicular dendritic cells in the pathway leading from immune complex formation to antigen presentation by germinal center B cells"

MARC FELDMANN
Charing Cross Sunley Research Center, Hammersmith, London, England
"The function of 'aberrant' Ia"

SESSION IV MOLECULES INVOLVED IN T CELL ACTIVATION
Chairperson: Arthur Weiss

Speakers

ARTHUR WEISS
University of California, San Francisco, CA
"The role of the T cell antigen receptor in T cell activation"

FRANK W. FITCH
University of Chicago, Chicago, IL
"Receptors on T cells: Actions and interactions"

ETHAN M. SHEVACH
National Institutes of Health, Bethesda, MD
"Alternative pathways of T cell activation"

GERALD R. CRABTREE
Stanford University Medical School, Stanford, CA
"Identification of nuclear target sequences for signals originating at the antigen receptor"

SESSION V SECOND SIGNALS IN T CELL ACTIVATION
Chairperson: John Imboden

Speakers:

JOHN IMBODEN
Veterans Administration Medical Center,
San Francisco, CA
"Signal transduction by T cell surface receptors"

PHYLLIS GARDNER
Stanford University Medical Center, Stanford, CA
"A calcium permeable channel involved in T lymphocyte activation"

RONALD SCHWARTZ
National Institutes of Health, Bethesda, MD
"Induction of a non-responsive state in IL-2 producing T cell clones"

WARREN J. LEONARD
National Institutes of Health, Bethesda, MD
"Molecular biology of the human Interleukin-2 receptor"

THE MECHANICS OF INTRACELLULAR TRAFFIC

Frances M. Brodsky, University of California San Francisco

Antigen presentation involves T cell recognition of processed antigen, bound to histocompatibility antigen. Processing generally includes antigen proteolysis and accumulated evidence suggests this takes place in an intracellular location. Degradation of internalized antigen probably occurs via the endocytic pathway, whereas degradation and processing of viral antigen is more complex, depending on whether it is a cytosolic or membrane-bound antigen. The question of where and how foreign and histocompatibility antigens contact each other in the cell is fundamental to understanding antigen presentation. Analysis of the intracellular trafficking process at a molecular level has provided some insights into how directed transport takes place between membrane compartments. Clathrin-coated vesicles are the vehicle for selective intracellular transport of membrane-bound receptors. Studies of clathrin structure have revealed self-assembling properties of the molecule which promote vesiculation as well as regions which may function in the targetting process. Inhibition of clathrin assembly in the cytoplasm, using anti-clathrin monoclonal antibodies, has shown a clear role for clathrin-coated vesicles in endocytosis and has helped to define a more specific role for clathrin-coated vesicles during biosynthesis. The fact that clathrin has been implicated in the biosynthesis as well as the endocytosis of histocompatibility antigens (HLA) suggests the possibility that HLA is directed to specialized compartments during these processes. Such compartments might promote association with processed, foreign antigen.

Pathways Followed By Cell Surface Glycoproteins After Endocytosis

Martin D. Snider
Department of Biochemistry
Case Western Reserve School of Medicine, Cleveland, OH

In recent years, studies on endocytosis in animal cells have concentrated on the receptor-mediated uptake of ligands by specific surface receptors. From these studies, it is now clear that ligand-receptor complexes enter cells and are transported to endosomes. In most cases ligands and receptors dissociate in the endosome; ligands are transported to lysosomes while the receptors return to the cell surface. Our work has concentrated on other, less well understood routes followed by surface proteins. In particular, the intersection of endocytic and exocytic membrane traffic has been studied by examining the recycling of membrane glycoproteins through the Golgi complex. These studies utilize Golgi enzymes of glycoprotein oligosaccharide synthesis. In these experiments, glycoproteins bearing oligosaccharides that are substrates for specific Golgi enzymes are generated in post-Golgi locations in cultured cells. Then, transport to enzyme-containing compartments is assessed by determining whether these immature glycoproteins have been converted to mature forms.

We have used this approach to examine glycoprotein recycling through compartments that contain sialyltransferase, a late Golgi enzyme and α-mannosidase I, an early Golgi enzyme. Studies on two surface receptors suggest that they cycle through the Golgi complex. One is the 215 kd mannose-6-phosphate receptor, which is involved in the packaging of lysosomal enyzmes into lysosomes and is also the receptor for insulin-like growth factor II. This glycoprotein returns to the Golgi complex from the cell surface within 1-2 hr. The second is transferrin receptor, which returns to the Golgi complex more slowly. In this case, recycling through the Golgi complex is clearly a secondary pathway, since surface transferrin receptor cycles through endosomes within 10-15 min during its function in iron uptake from serum transferrin.

Recently, we have found that a large number of the membrane glycoproteins that reside in post-Golgi compartments seem to cycle through the early Golgi compartment defined by α-mannosidase I. The half-time of this process (~4 hr) suggests that recycling glycoproteins represent a substantial fraction of the membrane glycoprotein flux through the Golgi complex. The importance of this recycling is unknown at present. Experiments to test several possible explanations are in progress.

THE TWENTY-SEVENTH MIDWINTER CONFERENCE OF IMMUNOLOGISTS

POSTER ABSTRACT: (Briefly summarize theme below)

Uptake and Processing of Proteins in Macrophage Endosomes

Receptor-mediated endocytosis is a complex process involving fusion of intracellular vesicles and selective targeting of products to different intracellular compartments. Macrophages express a cell surface receptor that recognizes and internalizes mannose-glycoproteins and mannose coated organisms by receptor-mediated endocytosis. The receptor is a 175 kD membrane glycoprotein which has been shown to recycle between the cell surface and acid intracellular compartments (i.e., endosomes). The mannose receptor has been used to study intracellular processing of ligands following endocytosis in two ways. First, mannose-BSA, has been shown to be rapidly taken up into cells via the mannose receptor. The internalized ligand is degraded within endosomes via the action of Cathepsin D. Cathepsin D has been localized in early endosomes by immunocytochemistry. Early processing of endocytosed proteins may provide a mechanism to generate biologically active peptides.

To reconstitute endocytosis in broken cell preparations a second approach has been taken. An in vitro fusion assay has been developed using two ligands that (i) recognize each other and (ii) are endocytosed via the mannose receptor. Mannosylated anti-DNP IgG and DNP-β-glucuronidase are rapidly taken up by separate populations of cells. Endosomes prepared from such cells are incubated together in the presence of an ATP regenerating system and cytosol. Endosome-ensome fusion is determined by the formation of anti-DNP-DNP-β-glucuronidase complexes, which are immunprecipitated following lysis of vesicles and quantitated using β-glucuronidase substrate. Fusion of endosomes requires cytosolic factors, K^+ and ATP.

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Processing and transport of MHC molecules

Peter Cresswell

Class II HLA antigens from human B-lymphoblastoid cell lines (LCL) are associated with two species in addition to the MHC-encoded α and β subunits during transport to the cell surface. These are the invariant (I) chain and a chondroitin sulfate proteoglycan (CSPG) which is an alternatively processed form of the I chain. The conventional I chain remains associated during transport through the Golgi apparatus but is absent from mature cell surface class II antigens. The fate of the CSPG following B cell surface expression is unknown. The work to be described will address the mechanism of I chain dissociation from class II antigens prior to cell surface expression, and the association of CSPG with cell surface class II antigens. Information concerning the mechanism of I chain dissociation comes from experiments examining the effects of the protease inhibitor leupeptin on class II antigen transport in B-LCL. Pulse-chase experiments indicate that in the presence of leupeptin a new 21 kd protein species is found associated with class II HLA antigens after approximately 2 hr of chase. This molecule (termed leupeptin-induced protein, Lip) appears to consist of a proteolytic fragment of the I chain. We hypothesize that the appearance of Lip results from inhibition of one step in the proteolytic degradation of the I chain which is normally responsible for its loss from maturing class II antigens. A corollary of this result is that class II antigens pass through a proteolytically active compartment on their way to the cell surface. This has implications for mechanisms of antigen processing. The fate of the CSPG has been studied by examining its' potential association with radioiodinated class II antigens. Experiments involving solubilization in the detergent CHAPS will be presented which indicate that at least 30% of cell surface HLA-DR antigens remain associated with the CSPG.

Structural requirements for binding of antigens to class II MHC molecules. Howard M. Grey, Alessandro Sette and Soren Buus.

One of the important questions that arises upon consideration of the hypothesis that Ia molecules interact specifically with foreign antigenic peptides to form immunogenic complexes that are in turn recognized by T cells is: How can a few Ia molecules interact with a very large universe of foreign antigens and yet maintain a degree of specificity to the interaction implied by Ir gene effects. We have approached this question by analyzing the capacity of antigenic peptides that differ by single amino acid substitutions from the known immunogenic peptide and determining the effect of such substitutions on the Ia binding. Five substitutions at each of 11 residues in the OVA peptide 325-335 were made and the capacity of these analogs to bind IA^d was compared with the native peptide by determining the capacity to inhibit the binding of ^{125}I OVA 323-339 to IA^d. The data indicate that most of the substitutions had no significant effect on the binding capacity of the peptide to IA^d; only 9 of 55 substitutions had a significant deleterious on binding activity (>3 fold) and only 7 substitutions lead to >5 fold decrease in binding capacity. The most dramatic decrease in binding involved 5 of the substitutions at residue V_{327} and A_{332}. The 2 non-conservative substitutions at each of these two positions lead to 10-85 fold reduction in binding activity. Less drastic effects on IA^d binding activity was also observed with non-conservative substitutions at residues H_{328} and E_{333}. These data indicate that the structural characteristics involved in the binding of antigen to Ia are such that a great deal of latitude in primary amino acid sequence is allowed. This is compatible with the requirement that a great many peptides, that are only marginally similar to one another, must all have the capacity to bind to a single Ia molecule.

Kinetics of Antigenic Peptide Association with MHC in Planar Membranes

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Kinetics of antigenic peptide binding to class II MHC molecules in planar membranes have been measured using fluorescence techniques, and also bioassays (triggering of antigenic peptide specific, class II MHC restricted T-helper hybridomas). Fluorescein-labeled OVA (323-339) - IA^d association and dissociation follows kinetics similar to those reported by Buus et al.[1] except for a fast component of dissociation found in the present study. Dissociation of fluorescein-labeled pidgeon cytochrome c peptide from IE^k is also biphasic. These cytochrome c 88-104 peptide-IE^k kinetics have also been measured using a bioassay. There is a marked inhibition of cytochrome c peptide association with IE^k in planar membranes by 1 - 10%, fetal calf serum, a result relevant to studies of energy transfer between fluorescein labeled peptides and MHC in planar membranes.[2]

(1) S. Buus, A. Sette, S. Colon, D. Jenis & H. Grey, Cell, 47, 1071-1077 (1986).
(2) T. Watts, H. Gaub & H.M. McConnell, Nature 47, 105-113 (1985).

VIRAL ANTIGEN PROCESSING AND PRESENTATION TO MHC CLASS I RESTRICTED T LYMPHOCYTES by T.J. Braciale, L. Morrison, M. Sweetser, D. Kittleson and V.L. Braciale.

An increasing body of evidence supports the view that both MHC class I and class II restricted T lymphocytes recognize processed forms of protein antigens. There is also emerging evidence that different pathways of antigen presentation may be employed preferentially in generating the MHC-antigen complexes recognized by class I and class II restricted T lymphocytes respectively. We will review current evidence for differences in antigen presentation to class I and class II T cells. We will also discuss recent data defining the antigenic sites on the influenza hemagglutinin recognized by class I restricted T lymphocytes and evidence for immunodominance in the sites recognized by class I restricted T lymphocytes. Finally, we will discuss the implications of these pathways differences for T lymphocyte recognition.

ANTIGEN-PRESENTING CELLS IN MHC CLASS II TRANSGENIC MICE

Carol Cowing, Linda Burkly, David Lo, Georg Widera, Pippa Marrack,
Carl Pinkert, Richard Palmiter, Ralph Brinster and Richard Flavell

An antigen-presenting cell must express class II genes of the MHC in order to activate $CD4^+$ helper T cells. These genes have restricted tissue expression and are under complex regulation. In order to characterize the regulation of class II genes and to understand their function in different cell types during ontogeny, we have introduced the $E\alpha^d$ MHC class II gene into (C57BL/6 x SJL) F2 mice, that fail to express their endogenous Eα genes due to a large deletion. Using the Eα structural gene with varying amounts of 5' non-coding DNA, we have generated unique lines of mice with limited constitutive and inducible expression of Eα mRNA and I-E protein.

Mice carrying Ea constructs with at least 2.0kb of 5' flanking DNA have normal expression of the gene. Between -1.4kb and -2.0kb there appears to be a region necessary for expression of the gene in B cells, as deletion of these sequences results in mice with normal tissue distribution of I-E except in B cells. Surprisingly, an Ea construct that includes only 0.75kb of DNA 5' of the ATG start site resulted once again in apparently normal expression of I-E, suggesting the presence of repressor sequences between -0.75 and -1.4kb. Mice carrying Ea constructs with 300bp of 5' flanking DNA have reduced expression in both B cell and monocyte/macrophage lineages. Sequences between -0.3 and -1.25kb appear devoid of positive regulatory elements, as deletion of this region from a construct starting at -2.0kb results in mice with a normal I-E protein phenotype.

Using transgenic mice expressing different I-E phenotypes, we are analyzing the immunologic function of class II genes in tolerance, restriction and antigen-presentation.

Relevant References
Pinkert et al. EMBO J. 4 : 2225-2230,1985.
Widera et al. Cell 51 :175-187,1987.

Summary of Sprent talk

The thymus has two main functions. First, T cells differentiating in the thymus show a marked propensity for recognizing foreign antigens in the context of "self" MHC molecules. Second, although T cells react strongly to allo MHC molecules, most T cells show tolerance to self MHC molecules. Evidence will be presented that these two properties of T cells are imprinted in the thymus by different cell types: epithelial cells select for the MHC-restricted specificity of T cells whereas self-tolerance is largely under the control of marrow-derived cells.

"The Role of Follicular Dendritic Cells in the Pathway leading from Immune Complex Formation to Antigen Presentation by Germinal Center B cells".

John G. Tew, M.H. Kosco and A.K. Szakal, Medical College of Virginia/VCU,Richmond.

A major objective of our work is to determine what happens to immunogen during the induction of a secondary response. In immune animals the immunogen encounters specific antibody and is rapidly converted into immune complexes. These complexes are quickly trapped, endocytosed and catabolized by macrophages. By 48 hours after immunization very little antigen (Ag) persists in or on these macrophages. However, some immune complex enters an alternative pathway which serves to preserve immunogen. Initially non-phagocytic cells transport immune complexes to follicular dendritic cells (FDC) deep in the lymph node outer cortex. The FDC dendrites "beaded" over the next few days and many of these beads were released. These beads represent immune complex coated bodies which we call "iccosomes". Iccosomes were trapped and endocytosed by germinal center (GC) B cells in the follicles. Presentation of the FDC derived Ag by the GC B cells was assessed by quantitation of IL-2 production using T cell hybridomas and IL-2 dependent cell lines. GC B cells are PNA+ and were isolated on a percoll gradient followed by a PNA panning procedure. In the absence of any exogenous Ag, GC B cells could induce IL-2 production. This continued for over a week after booster immunization. In contrast, no IL-2 production was stimulated by GC B cells taken two to three weeks after booster immunization when FDC were not releasing iccosomes. The results indicate that GC B cells process Ag obtained <u>in vivo</u> and are capable of presenting it to T cells. These data support the hypothesis that GC B cells play a role in the presentation of the FDC derived Ag to T cells <u>in vivo</u>. FDC regulate how much Ag is available and when it is available to GC B cells for processing and presentation.

MARC FELDMANN

CHARING CROSS SUNLEY RESEARCH CENTRE. LURGAN AVENUE. HAMMERSMITH. LONDON. W6

ROLE OF HLA CLASS II OVER EXPRESSION IN HUMAN AUTOIMMUNE DISEASES

In essentially all human autoimmune diseases, there is excessive expression of HLA class II, sometimes extending to cells which do not usually express class II, such as thyroid follicular cells. We termed this 'aberrant expression'. A key question is whether class II expression in tissues which do not normally express it is sufficient for the induction of autoimmunity. This was studied using thyrocytes infiltrating lymphoid cells in non toxic goitre.

Such thyrocytes could be induced with IFNγ to act as presenting cells, but these did not activate the infiltrating T cells. This is in contrast to Graves' disease where the infiltrating T cells were stimulated by autologous thyrocytes. Thus HLA expression while necessary is probably not sufficient.

We have used molecular methods to analyse class II mRNA production in rheumatoid arthritis. A high rate of synthesis was found, which persisted if the mixture of cells in a joint are placed in culture, without any extrinsic stimulation. This persistence was not due to a long half life of mRNA, but was due to cellular interactions leading to activation. Mutual interactions between T cells and APC thus seem to be at the root of many autoimmune diseases.

THE ROLE OF THE T CELL ANTIGEN RECEPTOR IN T CELL ACTIVATION

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Departments of Medicine and of Microbiology and Immunology
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The antigen receptor on most human T cells is comprised of a molecular complex of the α/β heterodimer and the 3-7 chains of CD3 (CD3/Ti). Whereas the function of the α/β heterodimer is to recognize antigen and major histocompatibility determinants, CD3 is thought to play a transmembrane signalling role. The evidence for this rests solely on the observation that anti-CD3 monoclonal antibodies (mAb) can function as agonists in activating T cells. Antigen, anti-receptor mAb or lectins initiate activation via the CD3/Ti complex by inducing the hydrolysis of phosphatidylinositol bis-phosphate (PIP_2) with consequent generation of inositol tris-phosphate and diacylglycerol. These two second messengers can induce an increase in cytoplasmic free calcium $[Ca^{2+}]_i$ and the activation of protein kinase C, respectively, events which have been causally related to cellular activation.

In an effort to relate the unusual structural features of the antigen receptor to its function as a signal transduction molecule, signalling mutants of the T cell leukemic line Jurkat have been derived. One such mutant, J.CaM 1, expresses normal levels of CD3/Ti but fails to mediate PIP_2 hydrolysis or increases of $[Ca^{2+}]_i$ in response to anti-Ti and some anti-CD3 mAb. Indirect evidence suggests that the site of the mutation within this cell lies within the proximal components of the signal transduction pathway but not within the Ti α or β chains. These and other studies suggest that conformational changes within the receptor complex may play a role in initiating signal transduction.

A number of other T cell surface molecules have been implicated in antigen-independent pathways of activation, mediating signal transduction and cellular activation events similar to those mediated by the CD3/Ti complex. In view of the similarity of the pathways involved and conflicting data regarding the dependency of these molecules upon CD3/Ti, we investigated the role of the antigen receptor in CD2-mediated activation. Utilizing antigen receptor deficient mutant cells, receptor reconstituted transfectants, and J.CaM 1, signalling and cellular activation via CD2 stimulation were shown to depend upon the expression of a functional CD3/Ti complex on the Jurkat line.

Receptors on T Cells: Actions and Interactions

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Clonal populations of T lymphocytes have facilitated the dissection of the complicated cellular events and interactions that follow stimulation with antigen. However, cause-effect relationships between a single well-defined stimulus and a specific, characterized receptor have proven to be more complicated than expected. For example, although antigen-specificity of T lymphocytes is determined by the clonally distributed proteins of the T cell receptor (TCR) (α/β or γ/δ), the closely associated CD3 complex appears to transduce the signal initiated by TCR stimulation. Also, the high affinity receptor for interleukin 2 (IL-2) consists of two protein chains, each of which binds IL-2 with low affinity.

In addition to these complexities, there are unexpected interactions among the various cell surface receptors on T lymphocytes: A) Exposure to IL-2 renders T cells which produce IL-2 unresponsive to stimulation through the TCR. B) Supra-optimal stimulation of the TCR induces unresponsiveness of T lymphocytes to IL-2 but does not affect IL-2-independent proliferation of T lymphocyte. C) Interferon-γ (IFN-γ) inhibits proliferation of at least one IL-1-dependent "T_H2"-type helper T lymphocyte (HTL) clone [D10] stimulated with either IL-4 or IL-2 but does not affect IL-2-dependent proliferation of "T_H1"-type HTL clones. These observations indicate that the immunoregulatory functions of lymphokines includes inhibitory as well as stimulatory effects. It appears that the particular array of lymphokines secreted by some T lymphocytes can direct which types of T lymphocytes increase in number during an immune response.

ALTERNATIVE PATHWAYS OF T CELL ACTIVATION

Ethan M. Shevach, Laboratory of Immunology, NIAID, NIH, Bethesda, MD

A number of cell surface molecules in addition to the CD3/TCR complex appear to play critical roles in T cell activation and it has been proposed that these structures may function as mediators of alternative pathways of T cell activation. We have extensively characterized the requirements for activation of murine T cells by monoclonal antibodies to Thy-1, Ly-6, and the murine homologue of human CD28. The activation pathways mediated by Thy-1 and Ly-6 are not truly independent as the induction of IL-2 production by anti-Thy-1 as well as anti-Ly-6 mAbs requires co-expression of the CD3/TCR complex. Both Thy-1 and Ly-6 are coupled to the cell membrane via a phosphatidyl-inositol (PI) linkage and both are removed from the cell surface by the enzyme PI-specific phopholipase C (PI-PLC). Treatment of normal T cells with PI-PLC abrogates their responsiveness to Con A, but does not affect responses to ionophore and PMA, or anti-CD3. Inhibition of the Con A response was secondary to removal of a PI-linked protein from the responder T cell population because PI-PLC treatment of accessory cells did not alter their function. Thus, Thy-1, Ly-6 or as yet uncharacterized PI-linked structures may play critical roles in the intercellular interactions required for certain T cell responses. The hamster mAb, H1.2F3, identifies the murine homologue of human CD28 and precipitates a dimer of 80 kd non-reduced and 40 kd reduced. H1.2F3 reacts with an antigen which is not expressed on resting T cells, but which is rapidly induced on all T and B cells by a short exposure to PMA. H1.2F3 is a potent inducer of T, but not B, cell proliferation in the presence of PMA and the stimulatory activity of the mAb appears to be restricted to $CD4^+$ T cells even though $CD4^+$ and $CD8^+$ T cells bear equivalent amounts of the antigen after activation. The molecule recognized by H1.2F3 is independent of the CD3/TCR complex in immunoprecipitation studies and the requirement for CD3/TCR expression for activation by H1.2F3 is presently under study.

Implication of Two Nuclear Proteins in the Antigen Receptor Dependent Activation of the IL-2 Gene

G.R. Crabtree, J.P. Shaw, P.J. Utz, K. Ullman, B. Emmel and D.B. Durand.
Department of Pathology Stanford University Medical School

We have used the antigen receptor-dependent activation of the IL-2 gene to begin to define the nuclear events responsible for directing T-cell activation. Previous studies have shown that the IL-2 gene is controlled by a transcriptional enhancer which functions only in activated T cells. An analysis of internal deletions of this enhancer revealed three sequences essential for maximal activation of a linked reporter gene. When synthetic oligonucleotides of two of these sequences are concatanated and linked to an unrelated promoter, either sequence renders the promoter responsive to signals from the antigen receptor. As found with the IL-2 enhancer, the ability of these oligonucleotides to activate a linked gene can be completely inhibited by 100 ng/ml cyclosporin A. These two oligonucleotides bind two different proteins, NFIL2A and NFIL2E. NFIL2A is constitutively expressed in all cells examined and binds to the SV40 enhancer. On the other hand NFIL2E is expressed only in activated T cells. The binding activity for NFIL2E appears by 20 minutes after T-cell activation and precedes IL-2 mRNA by 10 to 25 minutes. Inhibitors of protein synthesis inhibit the appearance of binding activity for NFIL2E and also inhibit IL-2 gene activation. Finally NFIL2E binds to essential regions of the promoters for other genes that are expressed during T-cell activation including the HIV LTR. For these reasons we feel that NFIL2E is involved in the early programing events in T-cell activation and propose the name NFAT-1 (Nuclear Factor of Activated T cells).

Transmembrane signaling by the T cell antigen receptor (CD3/Ti). John B. Imboden, Carl H. June, Michael McCutcheon, and Jeffrey A. Ledbetter. Signal transduction by CD3/Ti involves the hydrolysis of inositol phospholipids and an increase in the Ca^{2+}-mobilizing messenger, inositol-1,4,5-trisphosphate ($1,4,5IP_3$). We observe that a substantial proportion on the $1,4,5IP_3$ generated is in turn phosphorylated to inositol-1,3,4,5-tetrakisphosphate (IP_4), by the action of a soluble Ca^{2+}-regulated kinase. The Vmax of this kinase is increased as a result of stimulating CD3/Ti, indicating that the $1,4,5IP_3/IP_4$ pathway has a regulatory role during T cell activation. In Jurkat cells, sustained increases in $1,4,5IP_3$ are associated both with ongoing release of Ca^{2+} from intracellular stores and with extracellular Ca^{2+} influx, suggesting that $1,4,5IP_3$ directly or indirectly regulates $[Ca^{2+}]i$ due to Ca^{2+} influx.

Perturbation of the lymphocyte-specific cell-surface molecule CD5 (T1,Leu1, Tp67) can augment CD3/Ti-mediated activation of peripheral T cells. Using highly purified peripheral human T cells, we observe that monoclonal antibodies (MAB) to CD5 substantially augment CD3/Ti-mediated increases in the inositol phosphates. While CD5 MAb alone have no detectable effect on inositol phosphates, CD5-mediated increases in phosphatidylinositol phosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2) are observed in T cells labeled with $[^{32}P]$orthophosphate. Nonequilibrium labeling studies of Jurkat cells indicate that CD5 MAb stimulate a selective 33% increase in the rate of ^{32}P incorporation into PIP_2. CD5, therefore, may function as a accessory molecule in T cell activation by augmenting CD3/Ti-mediated generation of IPs, possibly through a primary effect on inositol phospholipid precursors.

Dr. Phyllis Gardner

Stimulation of human T-lymphocytes via mitogens or either of the surface structures, T3-Ti antigen/MHC receptor complex or T11 molecule, results in clonal proliferation through a calcium-dependent mechanism. To investigate the basis of this signal transduction, plasma membrane calcium channels were characterized in T-lymphocytes by means of whole cell or single channel patch clamp recordings. Stimulation of T-lymphocytes via any of the three mechanisms results in opening of an identical set of voltage-insensitive plasma membrane Ca^{2+} channels through the action of diffusable second messenger. Single channel recordings of excised patches suggested that inositol 1,4,5-trisphosphate ($InsP_3$), which is generated by phosphoinositide turnover after stimulation by mitogens and antibodies to both the T3-Ti and T11 structures, is the intermediary second messenger inducing Ca^{2+} channel opening. Furthermore, currents flowing through the Ca^{2+} permeable channels are apparently autoregulated as inward conductance is abrogated by elevation of Ca^{2+} concentration in the bathing solution. Importantly, IL-2 (T-cell growth factor) gene transcription is dependent upon the rise of $[Ca^{2+}]i$ resulting from ion movement across this channel.

EARLY MOLECULAR EVENTS IN THE INDUCTION OF A NONRESPONSIVE STATE IN TYPE I (INFLAMMATORY) $CD4^+$ T CELL CLONES. Marc K. Jenkins, Daniel Mueller and Ronald H. Schwartz, Laboratory of Cellular and Molecular Immunology, NIAID, NIH, Bethesda, MD 20892.

Antigen stimulation of normal T cell clones with antigen-presenting cells (APC) chemically modified with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI) results in unresponsiveness to subsequent stimulation with untreated APC and antigen as measured by both thymidine incorporation and interleukin-2 production. The unresponsive state lasts for more than one week, although the cells remain viable as manifested by their ability to respond to exogenous interleukin-2. Induction of unresponsiveness required new protein synthesis and was accompanied by the production of IL-3, IFN-γ, increases in TCRβ mRNA, and partial increases in IL-2 receptor expression; however, no IL-2 was produced. The critical biochemical event for the induction of unresponsiveness appeared to be a rise in intracellular calcium. Entry into the unresponsive state was blocked by EGTA and the state could be chemically induced by the addition of the calcium ionophore, ionomycin. Addition of allogeneic accessory cells during the exposure to ECDI-treated APC and antigen blocked the induction of nonresponsiveness and induced a proliferative response from the T cell clone. These effects could not be mimicked by addition of a phorbol ester and addition of the allogeneic cells did not increase hydrolysis of phosphatidylinositol polyphosphates nor did it activate protein kinase C. These observations suggest that occupancy of the antigen-specific receptor on IL-2 producing T cell clones, in the absence of any costimulatory signals, leads to an increase in intracellular calcium, activating a biochemical program that eventually prevents the cell from producing IL-2 in response to normal activation signals. If, however, an accessory cell costimulatory signal is present, the T cell clone divides and nonresponsiveness is prevented. The costimulatory signal does not appear to be transduced by activation of protein kinase C.

MOLECULAR BIOLOGY OF THE HUMAN INTERLEUKIN-2 RECEPTOR.

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IL-2 binds to either p55 (alpha chain) or p70 (beta chain), low and intermediate affinity forms of IL-2 receptors, respectively, and to high affinity alpha-beta heterodimers. Although high affinity IL-2 receptors mediate the mitogenic actions of IL-2 on activated T cells, the beta chain by itself appears to be responsible for IL-2 mediated boosting of NK activity of large granular lymphocytes (LGLs) and for the initiation of IL-2 induced proliferative and LAK activities of small resting T cells. Activation of such cells results in induction of the alpha chain and expression of high affinity receptors. In contrast to T cells and LGLs, resting B cells and monocytes do not express the beta chain. However, after activation of B cells with Staph aureus Cowan or monocytes with lipopolysaccharide plus Y-interferon, both alpha and beta chains are expressed.

The inducibility of the alpha chain after activation has led us to study the 5' regulatory sequences controlling the gene. Although the gene is transcribed in normal peripheral blood T cells only after activation, it is constitutively expressed in HTLV-I transformed T cells. Using IL2Rα-CAT constructs, we have previously shown that 5' flanking sequences regulate transcription both in cells that are inducible (Jurkat) or constitutive (HTLV-I transformed, e.g., MT-2) for IL2Rα expression, and we demonstrated that the HTLV-I encoded tat-I ($p40^{x}$, tax) gene in cotransfection experiments was capable of stimulating IL2Rα-CAT expression in Jurkat T cells. The 5' promoter sequences required for induction by tat-I and PMA are nonidentical. Extensive 5' and 3' deletion mutants are being analyzed, and indicate that the IL2Rα promoter requires several distinct regions for maximal activity. However, different regions are required in Jurkat cells than are necessary in MT-2 cells. We have also identiifed areas of protein binding in the 580 bp DNA fragment utilized in these studies.

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